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jc553 U.S. PTO 06/22/99

UTILITY PATENT APPLICATION TRANSMITTAL
(Large Entity)

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Docket No. Army 111A
Total Pages in this Submission

TO THE ASSISTANT COMMISSIONER FOR PATENTS
Box Patent Application
Washington, D.C. 20231

Transmitted herewith for filing under 35 U.S.C. 111(a) and 37 C.F.R. 1.53(b) is a new utility patent application for an invention entitled:

NOVEL "BURST-FREE" SUSTAINED RELEASE POLY-(LACTIDE/GLYCOLIDE) MICROPHERES

jc549 U.S. PTO 09/337945 06/22/99

and invented by:

Jeyanthi, et al.

If a CONTINUATION APPLICATION, *check appropriate box and supply the requisite information:*

☒ **Continuation** ☐ **Divisional** ☐ **Continuation-in-part (CIP)** of prior application No.: **08/590,973**

Which is a:

☐ **Continuation** ☐ **Divisional** ☒ **Continuation-in-part (CIP)** of prior application No.: **08/446,149**

Which is a:

☐ **Continuation** ☐ **Divisional** ☐ **Continuation-in-part (CIP)** of prior application No.: _____

Enclosed are:

Application Elements

1. ☒ Filing fee as calculated and transmitted as described below
2. ☒ Specification having **41** pages and including the following:
   - a. ☒ Descriptive Title of the Invention
   - b. ☒ Cross References to Related Applications *(if applicable)*
   - c. ☒ Statement Regarding Federally-sponsored Research/Development *(if applicable)*
   - d. ☐ Reference to Microfiche Appendix *(if applicable)*
   - e. ☒ Background of the Invention
   - f. ☒ Brief Summary of the Invention
   - g. ☒ Brief Description of the Drawings *(if drawings filed)*
   - h. ☒ Detailed Description
   - i. ☒ Claim(s) as Classified Below
   - j. ☒ Abstract of the Disclosure

UTILITY PATENT APPLICATION TRANSMITTAL
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(Only for new nonprovisional applications under 37 CFR 1.53(b))

Docket No. Army 111
Total Pages in this Submission

Application Elements (Continued)

3. ☒ Drawing(s) *(when necessary as prescribed by 35 USC 113)*
   a. ☐ Formal Number of Sheets _____
   b. ☒ Informal Number of Sheets 8
4. ☐ Oath or Declaration
   a. ☐ Newly executed *(original or copy)* ☐ Unexecuted
   b. ☒ Copy from a prior application (37 CFR 1.63(d)) *(for continuation/divisional application only)*
   c. ☒ With Power of Attorney ☐ Without Power of Attorney
   d. ☐ *DELETION OF INVENTOR(S)*
   Signed statement attached deleting inventor(s) named in the prior application, see 37 C.F.R. 1.63(d)(2) and 1.33(b).
5. ☒ Incorporation By Reference *(usable if Box 4b is checked)*
   The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.
6. ☐ Computer Program in Microfiche *(Appendix)*
7. ☐ Nucleotide and/or Amino Acid Sequence Submission *(if applicable, all must be included)*
   a. ☐ Paper Copy
   b. ☐ Computer Readable Copy *(identical to computer copy)*
   c. ☐ Statement Verifying Identical Paper and Computer Readable Copy

Accompanying Application Parts

8. ☒ Assignment Papers *(cover sheet & document(s))*
9. ☐ 37 CFR 3.73(B) Statement *(when there is an assignee)*
10. ☐ English Translation Document *(if applicable)*
11. ☐ Information Disclosure Statement/PTO-1449 ☐ Copies of IDS Citations
12. ☒ Preliminary Amendment
13. ☒ Acknowledgment postcard
14. ☒ Certificate of Mailing
    ☐ First Class ☒ Express Mail *(Specify Label No.):* EJ696804272US

UTILITY PATENT APPLICATION TRANSMITTAL
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(Only for new nonprovisional applications under 37 CFR 1.53(b))

Docket No. Army 111
Total Pages in this Submission

Accompanying Application Parts (Continued)

15. ☐ Certified Copy of Priority Document(s) *(if foreign priority is claimed)*

16. ☐ Additional Enclosures *(please identify below):*

Fee Calculation and Transmittal

CLAIMS AS FILED

For	#Filed	#Allowed	#Extra		Rate	Fee
Total Claims	46	- 20 =	26	x	$18.00	$468.00
Indep. Claims	4	- 3 =	1	x	$78.00	$78.00
Multiple Dependent Claims (check if applicable) ☐						$0.00
					BASIC FEE	$760.00
OTHER FEE *(specify purpose)* _____						
					TOTAL FILING FEE	$1,306.00

☐ A check in the amount of to cover the filing fee is enclosed.

☒ The Commissioner is hereby authorized to charge and credit Deposit Account No. **21-0380** as described below. A duplicate copy of this sheet is enclosed.

- ☒ Charge the amount of **$1,306.00** as filing fee.
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- ☒ Charge any additional filing fees required under 37 C.F.R. 1.16 and 1.17.
- ☐ Charge the issue fee set in 37 C.F.R. 1.18 at the mailing of the Notice of Allowance, pursuant to 37 C.F.R. 1.311(b).

Caroline Nash

Signature

Dated: **June 22, 1999**

Caroline M. Nash
Reg. No. 36, 329

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Brookeville, Maryland 20833
(301) 924-9500

cc:

CERTIFICATE OF MAILING BY "EXPRESS MAIL" (37 CFR 1.10) Applicant(s): **Jeyanthi, et al.**			Docket No. **Army 111A**
Serial No. **Cont. of 08/590,973**	Filing Date **June 22, 1999**	Examiner **Harrison, R.**	Group Art Unit **1617**

Invention: **NOVEL "BURST-FREE" SUSTAINED RELEASE POLY-(LACTIDE/GLYCOLIDE) MICROSPHERES**

I hereby certify that this **Assignment Recordation Form Cover Sheet, Transmittal Letter and Assignments (7)**
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is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 in an envelope addressed to: The Commissioner of Patents and Trademarks, Washington, D.C. 20231-0001 on **June 22, 1999**
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re PATENT APPLICATION of
JEYANTHI et al.
Rule 53(b) Continuation appl. Of
Appln. No.: 08/590,973
Filed: January 24, 1996

Group Art Unit: 1617

Examiner: Harrison, R.H.

FOR: NOVEL "BURST-FREE" SUSTAINED RELEASE POLY-(LACTIDE/GLYCOLIDE) MICROSPHERES

* * * * *

June 22, 1999

PRELIMINARY AMENDMENT

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

In connection with the above-referenced continuation application filed concurrently herewith, please enter the following amendments before examination on the merits.

IN THE SPECIFICATION:

Page 1, line 14, after "is" insert --- a continuation application of U.S. Patent Application Serial No. 08/590,973, filed January 24, 1996, which is --.

Page 6, line 20, delete "12k" second occurrence.

Page 8, line 19, replace "coploymer" with –copolymer--.

Page 12, line 16, after "13" insert –wherein the biologically active agent is a polypeptide--;

line 23, delete "of" first occurrence;

line 25, replace "microshreres" with --microspheres--.

Page 18, line 3, replace "preciptitous" with –precipitous--;

line 7, replace "yor" with –or--;

line 13, after "other' insert –biologically active agents from--.

Page 27, line 5, replace "microshperes" with –microspheres--;

line 22, after "1-12", replace "weeek" with –week--.

Page 28, line 14, replace "actice" with –active--;

line 20, replace "rations" with –ratios--;

line 50, replace "1a and 1b" with --2 and 4--.

Page 29, line 32, replace "2" with --5--;

line 49, replace "2" with --5--.

Page 30, line 13, replace "3" with--5--;

line 28, replace "2" with --3--;

line 43, replace "3" with ---5--.

Page 31, line 6, replace "3" with ---5--;

line 22, replace "2" with ---3--;

line 33, replace "is" with –are--;

line 34, replace "; and is" with --. They are--;

line 35, replace "4" with ---7--.

Page 32, line 20, replace "2 and 3" with --3 and 5--;

line 24, replace "these figures" with --Figure 6--.

IN THE CLAIMS:

Please amend the claims as follows:

1. (Amended) A controlled release microcapsule pharmaceutical formulation for burst-free, sustained, programmable release of a biologically active agent over a duration from 1-100 days, comprising an active agent and [a blend of] uncapped and end-capped biodegradable poly(lactide/glycolide) polymers.

2. (Amended) The microcapsule pharmaceutical formulation of claim 1, wherein [the biodegradable poly (lactide/glycolide) is a blend of] a copolymer ratio of uncapped and end-capped forms of biodegradable poly (lactide/glycolide) polymers [capped forms, in ratios ranging from] is 100/0 to 1/99.

3. (Amended) The [microcapsules] microcapsule pharmaceutical formulation of [claims 1 or 2] claim 2 wherein the copolymer (lactide to glycolide L/G) ratio for uncapped and [endcapped] end-capped polymer is 52/48 to 48/52.

4. (Amended) The [microcapsules] microcapsule pharmaceutical formulation of [claims 1 or 2] claim 2 wherein the copolymer (lactide to glycolide L/G) ratio for uncapped and end-capped polymer is 90/10 to 40/60.

5. (Amended) The [microcapsules] microcapsule pharmaceutical formulation of claim 3 wherein the molecular weight of the copolymer is between 2,000-60,000 daltons.

6. (Amended) The [microcapsules] microcapsule pharmaceutical formulation of claim 1 wherein the biologically active agent is a peptide or polypeptide.

7. (Amended) The [microcapsules] microcapsule pharmaceutical formulation of claim 6, wherein said polypeptide is histatin [consisting of 12 amino acids and having a molecular weight of 1563].

8. (Amended) The [microcapsules] microcapsule pharmaceutical formulation of claim 7 [characterized by the] having a capacity to completely release the histatin in an aqueous physiological environment from 1-35 days with a 100/0 [blend] ratio of uncapped and end-capped poly(lactide/glycolide) having an L/G ratio of 48/52 to 52/48, and a molecular weight <15,000 daltons.

9. (Amended) The [microcapsules] microcapsule pharmaceutical formulation of claim 7 [characterized by the] having a capacity to completely release the histatin in an aqueous physiological environment from 18-40 days with a 100/0 [blend] ratio of uncapped and end-capped poly(lactide/glycolide) having an L/G ratio of 48/52 to 52/48 and a molecular weight range of 28,000-40,000 daltons.

10. (Amended) The [microcapsules] microcapsule pharmaceutical formulation of claim 7 [characterized by the] having a capacity to release up to 90% of the histatin in an aqueous physiological environment from 28-70 days with a 0/100 [blend] ratio of uncapped and end-capped poly(lactide/glycolide) having a L/G ratio of 48/52 to 52/48 and a molecular weight range of 10,000 – 40,000 daltons.

11. (Amended) The [microcapsules] microcapsule pharmaceutical formulation of claim 7 [characterized by the] having a capacity to release up to 80% of the histatin in an aqueous physiological environment from 56-100 days with a 0/100 [blend] ratio of

uncapped and end-capped poly(lactide/glycolide) having a L/G ratio of 75/25 and a molecular weight of <15,000 daltons.

12. (Amended) The [microcapsules] microcapsule pharmaceutical formulation of [claims 7 or 8 or 9 or 10 or 11] claim 7 having analogs of histatin with chain lengths of from 11-24 amino acids of molecular weights from 1,500-3,000 daltons and characterized by the following structures:

1. D S H A K R H H G Y K R K F H E K H H S H R G Y
2. K R H H G Y K R K F H E K H H S H R G Y R
3. K R H H G Y K R K F H E K H H S H R
4. R K F H E K H H S H R G Y R
5. A K R H H G Y K R K F H
6. *A K R H H G Y K R K F H
7. K R H H G Y K R K F

*d-amino acid.

13. (Amended) The [microcapsules] microcapsule pharmaceutical formulation of claim 6 wherein the biologically active agent is a polypeptide Leutinizing hormone releasing hormone (LHRH) that is decapeptide of molecular weight 1182 in its acetate form, and having the structure:

p- E H W S Y G L R P G.

14. (Amended) The [microcapsules] microcapsule pharmaceutical formulation of claim 6 comprising a polypeptide, wherein said polypeptide has [having] a molecular weight of from 1,000 to 250,000 daltons.

15. (Amended) The [microcapsules] microcapsule pharmaceutical formulation of [claims 6 or 7 or 8 or 9 or 10 or 11 or 13 or 14] claim 1 wherein [release profiles of variable] varying release rates and varying release durations of the active agent are achieved by blending uncapped and [capped microspheres] end-capped polymers as a cocktail in variable amounts.

16. (Amended) The [microcapsules] microcapsule pharmaceutical formulation of [claims 6 or 7 or 8 or 9 or 10 or 11 or 13 or 14] claim 1 wherein [release of profiles of variable] varying release rates and varying release [duration] durations are achieved by varying a ratio of [blending] uncapped and [capped polymer] end-capped polymers [in different ratios] within the same [microshreres] microcapsules.

17. (Amended) The [microcapsules] microcapsule pharmaceutical formulation of claim 6 [or 7 or 8 or 9 or 10 or 11 or 13 or 14] wherein the [entrapped] active agent is a polypeptide and said polypeptide is [any of the] a vaccine [agents] agent against enterotoxigenic E. coli (ETEC) [such as CFA/I, CFA/II, CS1,CS3, CS6 and CS17 and other ETEC-related enterotoxins].

18. (Amended) The [microcapsules] microcapsule pharmaceutical formulation of claim 6 [or 7 or 8 or 9 or 10 or 11 or 13 or 14] wherein the [entrapped] active agent is a polypeptide and said polypeptide [consists of] comprises peptide antigens for immunization against enterotoxigenic E. coli (ETEC) [of] having a molecular weight range of about 800-5000 daltons [for immunization against enterotoxigenic E. coli (ETEC)].

19. (Amended) The [microcapsules] microcapsule pharmaceutical formulation of [claims 1 or 2 or 3 or 4 or 5] claim 1, wherein said biologically active [agents are] agent is selected from the group consisting of water-soluble hormone drugs, antibiotics, antitumor agents, anti inflammatory agents, antipyretics, analgesics, antitussives, expectorants, sedatives, muscle relaxants, antiepileptics, antiulcer agents, antidepressants, antiallergic drugs, cardiotonics, antiarrhythmic drugs, vasodilators, antihypertensives, diuretics, anticoagulants, and antinarcotics, in the molecular weight range of 100-100,000 daltons.

20. (Amended) The [microcapsules] microcapsule pharmaceutical formulation of [claims 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8] claim 1, wherein said biodegradable poly(lactide/glycolide) is in an oil phase, and is present in about 1-50% (w/w).

21. (Amended) The [microcapsules] microcapsule pharmaceutical formulation of [claims 1 or 2 or 3 or 4 or 5 or 6 or 8 or 9 or 10 or 11 or 12 or 13 or 14] claim 1, wherein a concentration of the active agent is in [the] a range of 0.1 to about 60% (w/w).

22. (Amended) The [microcapsules] microcapsule pharmaceutical formulation of [claims 1 or 2 or 3 or 4 or 5 or 6 or 8 or 9 or 10 or 11 or 12 or 13 or 14] claim 1, wherein a ratio of [the] an inner aqueous to oil [phases] phase is about 1/4 to 1/40 (v/v).

Claim 25, line 1, replace "claims 23 or 24" with ---claim 23---.

Claim 26, line 1, replace "claims 23 or 24" with ---claim 23---.

28. (Amended) The [microcapsules] microcapsule pharmaceutical formulation of claim 6, wherein, when the [entrapped] said active agent is a polypeptide and said polypeptide is active at a low pH and is entrapped in said polymers, [such as LHRH, adrenocorticortropic hormone, epidermal growth factor, calcitonin released] said polypeptide is bioactive when released.

29. (Amended) The [microcapsules] microcapsule pharmaceutical formulation of [claims 6 or 7 or 8 or 9 or 10 or 11] claim 6, wherein[,] said active agent is a peptide and when [entrapped] the peptide [such as histatin] is entrapped and is inactive at a low pH, a pH-stabilizing agent of inorganic salts [are] is added to [the] an inner aqueous phase during manufacture of said microcapsule to maintain biological activity of the released peptide.

30. (Amended) The [microcapsules] microcapsule pharmaceutical formulation of [claims 6 or 7 or 8 or 9 or 10 or 11] claim 6, wherein, when [entrapped] said active agent is a polypeptide, the polypeptide [such as histatin] is inactive at a low pH, a non-ionic surfactant [such as] selected from the group consisting of polyoxyethylene sorbitan fatty acid esters (Yween 80, Tween 60, and Tween 20) and polyoxyethylene – polyoxypropylene block copolymers (Pluronics) is added to [the] an inner aqueous phase during manufacture to maintain biological activity of the released polypeptide.

31. (Amended) The [microcapsules] microcapsule pharmaceutical formulation of claim 29, wherein placebo [spheres] microcapsules loaded with the pH-stabilizing agents are coadministered with the polypeptide-loaded [spheres] microcapsules to maintain [the] a solution pH around the polypeptide-loaded microcapsules and preserve [the] biological

activity of [the] released peptides [peptide in instances where the addition of pH-stabilizing agents in the inner aqueous phase is undesirable for the successful encapsulation of the acid pH sensitive polypeptide].

32. (Amended) The [microcapsules] microcapsule pharmaceutical formulation of claim 29, wherein placebo [spheres] microcapsules loaded with non-ionic surfactant are coadministered with polypeptide-loaded microcapsules [spheres] to maintain biological activity of released peptides [peptide where the addition of non-ionic surfactants in the inner aqueous phase is undesirable for successful encapsulation of the acid pH sensitive polypeptide].

33. (Amended) The [microcapsules] microcapsule pharmaceutical formulation of [claims 1 or 2 or 3 or 4 or 5 or 6 or 8 or 9 or 10 or 11 or 13 or 14] claim 2, wherein [comprising a blend of] said uncapped and [capped] end-capped polymers [polymer, wherein] are present in an amount wherein complete solubilization of the copolymer leaves no residual [polymer] polymers at [the] a site of administration and occurs concurrently with [the] a complete release of the [entrapped] active agent from said microcapsule.

34. A process for ameliorating or preventing a disease or disorder in a human or nonhuman animal comprising administering parenterally to said animal a pharmaceutically-effective amount of a microcapsule of claim 1 [of using microcapsules of claims 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 13 or 14 for human administration via parenteral routes, such as intramuscular and subcutaneous].

35. (Amended) A process for ameliorating or preventing a disease or disorder in a human or nonhuman animal comprising administering topically to said animal a pharmaceutically-effective amount of a microcapsule of claim 1 [of using microcapsules of claims 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 13 or 14 for human administration via topical routes].

36. (Amended) A process for ameliorating or preventing a disease or disorder in a human or nonhuman animal comprising administering orally to said animal a pharmaceutically-effective amount of a microcapsule of claim 1 [of using microcapsules of items 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 13 or 14 for human administration via oral routes].

37. (Amended) A process for ameliorating or preventing a disease or disorder in a human or nonhuman animal comprising administering nasally, rectally, transdermally or vaginally to said animal a pharmaceutically-effective amount of a microcapsule of claim 1 [of using microcapsules of items 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 13 or 14 for human administration via nasal, transdermal, rectal, and vaginal routes].

Please add the following claims:

--38. (New) The microcapsule pharmaceutical formulation of claim 17, wherein said enterotoxigenic E. coli is selected from the group consisting of CFA/I, CFA/II, CS1,CS3, CS6 and CS17 and other ETEC-related enterotoxins.

39. (New) The microcapsule pharmaceutical formulation of claim 28, wherein said polypeptide is selected from the group consisting of LHRH, adrenocorticortropic hormone, epidermal growth factor and calcitonin.

40. (New) The process of claim 34, wherein said administering parenterally comprises administering intramuscularly or subcutaneously.

41. (New) A process of maintaining a solution pH around the polypeptide-loaded microcapsule of claim 6, comprising coadministering placebo microcapsules loaded with pH-stabilizing agents with the polypeptide-loaded microcapsules, wherein said placebo microcapsules preserve biological activity of peptides released from said polypeptide loaded microcapsules.

42. (New) A process of maintaining biological activity of released peptide comprising coadministering placebo microcapsules loaded with non-ionic surfactant with said microcapsule pharmaceutical formulation of claim 29.

43. (New) The microcapsule pharmaceutical formulation of claim 7, wherein said histatin consists of 12 amino acids and has a molecular weight of 1563.

44. (New) A controlled release microcapsule pharmaceutical formulation for burst-free sustained, programmable release of biologically active agent over a duration from 1-100 days, comprising an active agent encapsulated within a biodegradable poly(lactide/glycolide) having a lactide/glycolide ratio of 90/10 to 40/60 and the end capped/uncapped form of said poly(lactide/glycolide) in the ratio of 100/0 to 1/99.

45. (New) The process of claim 24, wherein a solvent-saturated external aqueous phase is added to emulsify the inner w/o emulsion prior to addition of the external

aqueous layer, to provide microcapsules of narrow size distribution range between 0.05-500μm.

46. (New) The process of claim 24, wherein a low temperature of about 0-4°C is provided during preparation of the inner w/o emulsion, and a low temperature of about 4-20°C is sprovided during preparation of the w/o/w emulsion to provide a stable emulsion and high encapsulation efficiency.--.

REMARKS

An early and favorable action on the merits is respectfully requested.

Claims 1-46 are pending in the application. Claims 1-22, 25, 26 and 28-37 have been amended. Claims 38-46 are newly added. The specification has also been amended to correct various spelling, grammatical and idiomatic errors. No new matter has been added.

The claims have been amended to specifically address the Examiner's comments and section 112 rejections in the office actions of the parent application 08/590,973.

Additionally, the terms "uncapped" and "end-capped" are defined in the specification at page 8, lines 5-18.

The Examiner indicated in the parent application (08/590,973) that claim 38 was allowable. Claim 38 of the parent application (08/590,973) appears in this Preliminary Amendment as new claim 44. Hence, it is believed that new claim 44 would also be allowable.

If the Examiner has any questions, or would like to suggest alternative claim language, he is invited to contact Applicant's representative at (301) 924-9500.

Respectfully submitted,

By 

Caroline Nash, Reg. No. 36, 329
for: Charles H. Harris, Reg. No. 34,616
Attorney for Applicants
U.S. Army Medical Research
and Materiel Command
ATTN: MCMR-JA
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NOVEL, "BURST-FREE" SUSTAINED RELEASE POLY-(LACTIDE/GLYCOLIDE) MICROSPHERES

I. GOVERNMENT INTERESTS

The invention described herein may be manufactured, licensed and used by or for governmental purposes without the payment of any royalties to us thereon.

II. CROSS REFERENCE

This application is a continuation-in-part of U.S. Patent application Serial No. 08/446,149, filed May 22, 1995.

III. FIELD OF THE INVENTION

This invention relates to providing novel biocompatible and biodegradable microspheres for burst-free programmable sustained release of biologically active agents, inclusive of polypeptides, over a period of up to 100 days in an aqueous physiological environment.

IV. BACKGROUND OF THE INVENTION

Several publications and patents are available for sustained release of active agents from biodegradable polymers, particularly, poly(lactide/glycolides) (PLGA). Prior usages of PLGA for controlled release of polypeptides have involved the use of molar ratios of lactide/glycolide (L/G) of 75/25 to 100/0 for molecular weights >20,000. Further prior art preparations of PLGA utilized fillers or additives in the inner aqueous layer to improve the stability and encapsulation efficency and/or to increase the viscosity of the aqueous layer, thereby modulating polymer hydrolysis and the biologically active agent or polypeptide release.

In addition, the prior art use of PLGA copolymers were end-capped, in that the terminal carboxyl end groups were blocked. In these end-capped co-polymers, the microcapsule preparations exhibited a low to moderate burst release of ~ 10-40% of the entrapped polypeptide in the first 24 hours after placement in an aqueous physiological environment. In part, these characteristics are due to the use of fillers in the inner aqueous phase. Further, a 1-month release of polypeptide is known with the use of a 75/25 co-polymer of PLGA of Mw <20,000.

Investigations in controlled release research has been proceeding especially to obtain a 1 to 2 month delivery system

for biologically active agents or polypeptides using poly(lactide/glycolide) polymers. However, most of these systems have one or more of the following problems: Poor encapsulation efficency and large 'burst release' followed by an intermediate 'no release' or 'lag phase' until the polymer degrades. In general, release from these polymers occur over a period from about 4 weeks to about several months. In addition, in order to achieve this release a 50/50 copolymer of MW > 30,000 or a 75/25 copolymer of Mw > 10,000 are employed which often results in residual polymer remaining at the site of administration long after the release of active core.

V. SUMMARY OF THE INVENTION

This invention provides biocompatible and biodegradable microspheres that have been designed for novel, burst free, programmable sustained release of biologically active agents, including polypeptides over a period of up to 100 days in an aqueous physiological environment.

Unlike currently available release systems, which rely on the use of fillers/additives such as gelatin, albumin, dextran, pectin, polyvinyl pyrrolidone, polyethylene glycol, sugars, etc., and are still prone to low encapsulation efficiencies and "burst effects", this invention achieves high encapsulation and "burst-free" release without the use of any additive. In this invention, burst-free, programmable sustained release is achieved through the use of a unique blend of the 'uncapped' and end-capped forms of poly(lactide/glycolide) polymer in the molecular weight range of 2,000 to 60,000 daltons.

In general, microspheres described in this invention are produced by a unique emulsification technique wherein an inner water-in-oil (w/o) emulsion is stabilized by dispersing in a solvent-saturated aqueous phase containing an emulsion stabilizer. A ternary w/o/w emulsion is then formed by emulsifying the above w/o emulsions in an external pre-cooled aqueous phase containing an o/w emulsifier. Essentially, the

inner w/o emulsion is comprised of an aqueous layer containing from ~ 2 to about 20% (w/w) of the active agent to be entrapped and an oil layer containing poly(lactide/glycolide) copolymer in concentrations ranging from ~ 5 to about-- 50% (w/w oil phase). The copolymer includes molecular weight ranging from 2,000 to about 60,000 daltons, with molar composition of lactide/glycolide from 90/10 to 40/60 and a blend of its uncapped and end-capped forms in a ratio of 100/0 to 1/99. Very high encapsulation efficiencies of about 80 to 100% are achieved depending on polymer molecular weight and structural form.

Programmable release of active core over variable durations between 1-100 days is achieved by a judicious selection of process parameters such as polymer concentration, peptide concentration and the aqueous/oil phase ratio.

This invention is particularly suitable for high encapsulation efficiencies and burst-free, continuous programmable release of polypeptides of molecular weights ranging from 1,000 to about 250,000 daltons, and also other biologically active agents over a period of 1-100 days. A uniqueness of the invention is that when using a 100/0 blend of the uncapped and capped polymer, the final phase of active core release is concurrent with the complete solubilization of the polymer to innocuous components, such as lactic and glycolic acids. This is a significant advantage over the currently available 30 day - release systems wherein a major regulatory concern is about toxicity of residual polymer at the site of administration, long

after release of the active core.

The microcapsules described in this invention are suitable for administration via several routes such as parenteral (intramuscular, subcutaneous), oral, topical, nasal, rectal and vaginal routes.

VI. BRIEF DESCRIPTION OF DRAWINGS

FIG. 1 shows a comparison of drug release from a conventional system versus a controlled release system. Peak and valley levels from conventional administrations are shown, in contrast to the steady therapeutic levels from the controlled release administration.

FIG. 2 shows a scanning electron micrograph of PLGA microspheres prepared by the process described in the invention using 50/50 uncapped polymer of Mw 8-12k dalton and shows superior sphere morphology, sphere integrity, and narrow size distribution.

FIG. 2a shows a scanning electron micrograph of PLGA microspheres prepared by conventional solvent evaporation method using a 50/50 12k uncapped polymer of Mw 8-12k dalton.

FIG. 3 shows cumulative Histatin release from PLGA microspheres, wherein release profiles from several batches are prepared using 50/50, uncapped polymer (of Mw 8-12k dalton) and wherein the process parameters are varied to modulate release between 1 and 35 days.

FIG. 4 shows a scanning electron micrograph of solid, smooth

spherical surfaces of PLGA microspheres prepared by the method of in the invention using 50/50, end-capped polymer (of Mw 30-40k dalton).

FIG. 5 shows cumulative Histatin release from PLGA microspheres, wherein the release profiles are from several batches prepared using 50/50, uncapped and end-capped polymer of Mw 30-40k daltons, and wherein the process parameters are varied to modulate release between 28 to 60 days.

FIG. 6 shows cumulative Histatin release from PLGA microspheres, wherein combined release profiles from several batches have been prepared using 50/50, uncapped and end-capped polymer of Mw 8-40k daltons, while varying the process parameters to modulate release between 1 and 60 days.

FIG. 7 shows a cumulative percent release of LHRH from PLGA microspheres prepared using uncapped polymer of Mw 8-12 daltons.

DETAILED DESCRIPTION OF THE INVENTION

This invention relates to the design of biocompatible and biodegradable microspheres for novel, programmable sustained release of biologically active agents, including polypeptides over a period of up to 100 days in an aqueous physiological environment with little or no burst release.

Unlike currently available release systems which rely on the use of fillers/additives such as gelatin, albumin, dextran, pectin, polyvinyl pyrrolidone, polyethylene glycol, sugars, etc., and are still prone to low encapsulation efficiencies and "burst effects", this invention achieves high encapsulation efficiency

and 'burst-free' release without the use of any additive. In this invention, burst-free, programmable sustained release is achieved through the use of a unique blend of the 'uncapped' and end-capped forms of poly (lactide/glycolide) polymer.

The 'uncapped' form refers to "poly(lactide/glycolide) with free carboxyl end groups" which renders the polymer more hydrophilic compared to the routinely used end-capped form. Currently used 'end-capped' polymer hydrates between 4-12 weeks depending on the molecular weight, resulting in an intermediate 'no release' or a 'lag phase'. The uncapped polymer hydrates typically between 5 to 60 days depending on the molecular weight, thus releasing its core continuously without a lag phase. A careful blend of the two forms and appropriate molecular weights and L/G ratios, results in a continuous release between 1 to 100 days. In addition, release within this time is programmable by a judicious selection of process parameters such as polymer concentration, peptide concentration and the aqueous/oil phase ratio.

The coploymer in this invention includes molecular weight ranging from 2,000 to 60,000 daltons, a lactide/glycolide ratio of 90/10 to 40/60 and a blend of the uncapped/capped forms in the ratio of 100/0 to 1/99. The molecular weight of the polypeptide may be in the range of 1000 to 250,000 daltons while that of other biologically active agents may range from 100 to 100,000 daltons.

Microcapsules described in this invention are prepared by a

unique aqueous emulsification techinique which has been developed for use with the uncapped polymer to provide superior sphere morphology, sphere integrity and narrow size distribution. This is accomplished by first preparing an inner water-in-oil (w/o) by mixing the solutions of polymer in an organic solvent such as methylene chloride and the biologically active agent in water. This is followed by stabilization of the w/o emulsion in a solvent-saturated aqueous solution containing an o/w emulsifier such as polyvinyl alcohol. A ternary emulsion is then formed by emulsifying the w/o emulsion in an external aqueous phase containing the same emulsifier as above at concentrations ranging from 0.25 - 1% w/v. Microcapsules are hardened upon solvent removal by evaporation, rinsed to remove residual emulsifier and lyophilized. Low temperature is used both at the time of primary emulsification (w/o emulsion formation) and during the formation of the final w/o/w emulsion to achieve stable emulsion and superior sphere characteristics.

In the context of the invention, a biologically active agent is any water-soluble hormone drugs, antibiotics, antitumor agents, antiinflammatory agents, antipyretics, analgesics, antitussives, expectorants, sedatives, muscle relaxants, antiepileptics, antiulcer agents, antidepressants, antiallergic drugs, cardiotonics, antiarrhythmic drugs, vasodilators, antihypertensives, diuretics, anticoagulants, antinarcotics, etc.

More precisely, applicants have discovered a pharmaceutical composition and process with the following itemized features:

1. A controlled release microcapsule pharmaceutical formulation for burst-free, sustained, programmable release of a biologically active agent over a duration from 1-100 days, comprising an active agent and a blend of uncapped and end-capped biodegradable poly(lactide/glycolide).

2. The pharmaceutical formulation of item 1, wherein the biodegradable poly(lactide/glycolide) is a blend of uncapped and capped forms, in ratios ranging from 100/0 to 1/99.

3. The microcapsules of items 1 or 2 wherein the copolymer (lactide to glycolide L/G) ratio for uncapped and endcapped polymer is 52/48 to 48/52.

4. The microcapsules of items 1 or 2 wherein the copolymer L/G ratio for uncapped and end-capped polymer is 90/10 to 40/60.

5. The microcapsules of items 1 or 2 or 3 or 4 wherein the molecular weight of the copolymer is between 2,000-60,000 daltons.

6. The microcapsules of items 1 or 2 or 3 or 4 or 5 wherein the biologically active agent is a peptide or polypeptide.

7. The microcapsules of item 6, wherein said polypeptide is histatin consisting of 12 amino acids and having a molecular weight of 1563.

8. The microcapsules of items 1 or 2 or 3 or 4 or 5 or 6 characterized by the capacity to completely release histatin in

an aqueous physiological environment from 1-35 days with a 100/0 blend of uncapped and end-capped poly(lactide/glycolide) having a L/G ratio of 48/52 to 52/48, and a molecular weight <15,000.

9. The microcapsules of items 1 or 2 or 3 or 4 or 5 or 6 characterized by the capacity to completely release histatin in an aqueous physiological environment from 18-40 days with a 100/0 blend of uncapped and end-capped poly(lactide/glycolide) having a L/G ratio of 48/52 to 52/48 and a molecular weight range of 28,000-40,000.

10. The microcapsules of items 1 or 2 or 3 or 4 or 5 or 6 characterized by the capacity to release up to 90% of the histatin in an aqueous physiological environment from 28-70 days with a 0/100 blend of uncapped and end-capped poly(lactide/glycolide) having a L/G ratio of 48/52 to 52/48 and a molecular weight range of 10,000-40,000 daltons.

11. The microcapsules of items 1 or 2 or 3 or 4 or 5 or 6 characterized by the capacity to release up to 80% of histatin in an aqueous physiological environment from 56-100 days with a 0/100 blend of uncapped and end-capped poly(lactide/glycolide) having a L/G ratio of 75/25 and a molecular weight of < 15,000 daltons.

12. The microcapsules of items 7 or 8 or 9 or 10 or 11 having analogs of histatin with chain lengths of from 11-24 amino acids of molecular weights from 1,500-3,000 daltons and characterized by the following structures:

1. D S H A K R H H G Y K R K F H E K H H S H R G Y

2. K R H H G Y K R K F H E K H H S H R G Y R

3. K R H H G Y K R K F H E K H H S H R

4. R K F H E K H H S H R G Y R

5. A K R H H G Y K R K F H

6. *A K R H H G Y K R K F H

7. K R H H G Y K R K F

* D-amino acid

13. The microcapsules of items 1 or 2 or 3 or 4 or 5 wherein the biologically active agent is a polypeptide Leutinizing hormone releasing hormone (LHRH) that is a decapeptide of molecular weight 1182 in its acetate form, and having the structure:

p- E H W S Y G L R P G

14. The microcapsule of items 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 having a molecular weight of from 1,000 to 250,000 daltons.

15. The microcapsules of items 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 wherein release profiles of variable rates and durations are achieved by blending uncapped and capped microspheres as a cocktail in variable amounts.

16. The microcapsules of items 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 wherein release of profiles of variable rates and duration are achieved by blending uncapped and capped polymer in different ratios within the same microshreres.

17. The microcapsules of items 6 or 7 or 8 or 9 or 10 or 11

or 12 or 13 or 14 or 15 or 16 wherein the entrapped polypeptide is any of the vaccine agents against enterotoxigenic E. coli (ETEC) such as CFA/I,CFA/II,CS1,CS3,CS6 and CS17 and other ETEC-related enterotoxins.

18. The microcapsules of items 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 wherein the entrapped polypeptide consists of peptide antigens of molecular weight range of about 800-5000 daltons for immunization against enterotoxigenic E. coli (ETEC).

19. The microcapsules of items 1 or 2 or 3 or 4 or 5 wherein said biologically active agents are selected from the group consisting of water-soluble hormone drugs, antibiotics, antitumor agents, anti inflammatory agents, antipyretics, analgesics, antitussives, expectorants, sedatives, muscle relaxants, antiepileptics, antiulcer agents, antidepressants, antiallergic drugs, cardiotonics, antiarrhythmic drugs, vasodilators, antihypertensives, diuretics, anticoagulants, and antinarcotics, in the molecular weight range of 100-100,000 daltons.

20. The microcapsules of items 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 wherein said biodegradable poly(lactide/glycolide) is in an oil phase, and is present in about 1-50% (w/w).

21. The microcapsules of items 1 or 2 or 3 or 4 or 5 or 6 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 wherein concentration of the active agent is in the range of 0.1 to about 60% (w/w).

22. The microcapsules of items 1 or 2 or 3 or 4 or 5 or 6 or

8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 wherein a ratio of the inner aqueous to oil phases is about 1/4 to 1/40(v/v).

23. A process for preparing controlled release microcapsule formulations characterized by burst-free, sustained, programmable release of biologically active agents comprising: Dissolving biodegradable poly (lactide/glycolide), in uncapped form in methylene chloride, and dissolving a biologically active agent or active core in water; adding the aqueous layer to the polymer solution and emulsifying to provide an inner water-in-oil (w/o) emulsion; stabilizing the w/o emulsion in a solvent-saturated aqueous phase containing a oil-in-water (o/w) emulsifier; adding said w/o emulsion to an external aqueous layer containing oil-in-water emulsifier to form a ternary emulsion; and stirring the resulting water-in-oil-in-water (w/o/w) emulsion for sufficient time to remove said solvent, and rinsing hardened microcapsules with water and lyophilizing said hardened microcapsules.

24. A process for preparing controlled release microcapsule formulations characterized by burst-free, sustained, programmable release of biologically active agents comprising:

dissolving biodegradable poly(lactide/glycolide) in end-capped form in methylene chloride, and dissolving a biologically active agent or active core in water; adding the aqueous layer to the polymer solution and emulsifying to provide an inner water-in-oil emulsion; stabilizing the w/o emulsion in a solvent-saturated aqueous phase containing a oil-in-water (o/w)

emulsifier; adding said w/o emulsion to an external aqueous layer containing oil-in-water emulsifier to form a ternary emulsion; and stirring a resulting water-in-oil-water (w/o/w) emulsion for sufficient time to remove said solvent; and rinsing hardened microcapsules with water; and lyophilizing said hardened microcapsules.

25. The process of items 23 or 24 wherein a solvent-saturated external aqueous phase is added to emulsify the inner w/o emulsion prior to addition of the external aqueous layer, to provide microcapsules of narrow size distribution range between 0.05-500μm.

26. The process of items 23 or 24, wherein a low temperature of about 0-4°C is provided during preparation of the inner w/o emulsion, and a low temperature of about 4-20°C is provided during preparation of the w/o/w emulsion to provide a stable emulsion and high encapsulation efficiency.

27. The process of items wherein a 100/0 blend of uncapped and end-capped polymer is used to provide release of the active core in a continous and sustained manner without a lag phase.

28. The microcapsules of items 6, wherein, when the entrapped polypeptide is active at a low pH, such as LHRH, adrenocorticotropic hormone, epidermal growth factor, calcitonin released polypeptide is bioactive.

29. The microcapsules of items 6 or 7 or 8 or 9 or 10 or 11, wherein, when entrapped peptide such as histatin is inactive at a low pH, a pH-stabilizing agent of inorganic salts are added to

the inner aqueous phase to maintain biological activity of the released peptide.

30. The microcapsules of items 6 or 7 or 8 or 9 or 10 or 11 wherein, when entrapped polypeptide such as histatin is inactive at a low pH, a non-ionic surfactant such as polyoxyethylene sorbitan fatty acid esters (Tween 80, Tween 60 and Tween 20) and polyoxyethylene - polyoxypropylene block copolymers (Pluronics) is added to the inner aqueous phase to maintain biological activity of the released polypeptide.

31. The microcapsules of items 29, wherein placebo spheres loaded with the pH-stabilizing agents are coadministered with polypeptide-loaded spheres to maintain the solution pH around the microcapsules and preserve the biological activity of the released peptide in instances where the addition of pH-stabilizing agents in the inner aqueous phase is undesirable for the successful encapsulation of the acid pH sensitive polypeptide.

32. The microcapsules of item 30 wherein placebo spheres loaded with non-ionic surfactant are coadministered with polypeptide-loaded spheres to maintain biological activity of the released peptide where the addition of non-ionic surfactants in the inner aqueous phase is undesirable for successful encapsulation of the acid pH sensitive polypeptide.

33. The microcapsules of items 1 or 2 or 3 or 4 or 5 or 6 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 comprising a blend of uncapped and capped polymer, wherein complete

solubilization of the copolymer leaves no residual polymer at the site of administration and occurs concurrently with the complete release of the entrapped agent.

34. A process of using microcapsules of items 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 for human administration via parenteral routes, such as intramuscular and subcutaneous.

35. A process of using microcapsules of items 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 for human administration via topical route.

36. A process of using microcapsules of items 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 for human administration via oral routes.

37. A process of using microcapsules of items 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 for human admininstration via nasal, transdermal, rectal, and vaginal routes.

Conservation of bioactivity of polypeptides

As the polymer degrades rapidly, there is a preciptitous drop in pH accompanied by the release of soluble oligomers in the microenvironment which may affect the biological activity of acid pH-sensitive peptides/proteins. In such instances, biological activity can be maintained by the use of inorganic salts yor buffering agents in the inner aqueous phase codissolved with the peptide.

The following unique advantages are characteristics of this invention:

1. Burst-free, prolonged, sustained release of polypeptides and other biocompatible and biodegradable microcapsules up to 100 days in an aqueous physiological environment without the use of additives in the core.

2. Release of active core programmable for variable durations over 1-100 days, by using a blend of uncapped and capped polymer of different molecular weights and copolymer ratio, and by manipulating the process parameters.

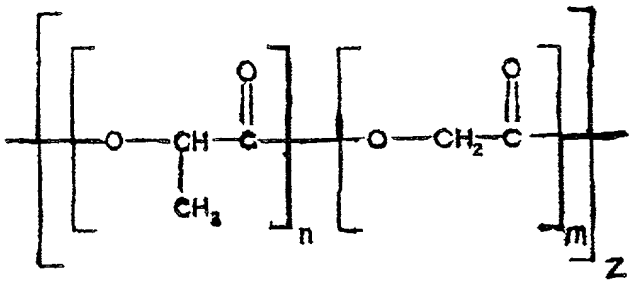
3. Complete release of the active core is concurrent with complete solubilization of the carrier polymer to innocuous components, such as lactic and glycolic acids, especially when using a 100/0 blend of uncapped/capped polymer. This is of tremendous significance, as most biodegradable polymers currently used for 1-30 day delivery, do not degrade completely at the end of the intended release duration, thereby causing serious concern

of regulatory authorities on the effects of residual polymer at the site of administration.

4. Ease of administration of the microcapsules in various dosage forms via several routes, such as parenteral (intramuscular and sucutaneous), oral, topical, nasal, vaginal, etc.

The hydrophilic homo-and co-polymers based on D,L-lactide and glycolide contains hydrophilic adjusted homo-and co-polymers with free carboxylic end groups, and is characterized by the formula:

Poly(D,L-lactide-co-glycolide) 50:50



$(C_3H_4O_2)_n(C_2H_2O_2)_m$ n:m = 1:1

Wherein Z= Molecular Weight/130; for example Z=92 for Mw 12,000 and 262 for Mw 34,000.

While the molar ratio of the lactide to glycolide may vary, it is most preferred that the lactide to glycolide copolymer ratio be 50:50.

Reference is now made to FIG. 1 which depicts a blood-drug concentration versus time graph that shows conventional drug administration using a series of dosages compared to an ideal controlled release system. Unfortunately, many drugs have a

therapeutic range, above which they are toxic and below which they are ineffective. Oscillating drug levels that are commonly observed following systemic administration causes alternating periods of ineffectiveness and toxicity. A sustained-release encapsulated biologically active agent or polypeptide preparation, ideally, will maintain the drug in the desired therapeutic range by means of a single dose, as depicted in the THERAPEUTIC RANGE in FIG.1 , where the ideal case for controlled release is shown.

In FIG. 2, there is shown a scanning electron micrograph of PLGA microspheres prepared using 50/50 uncapped polymer of Mw 8-12k dalton. The uncapped polymer has solid, smooth spherical surfaces, and is suited to provide a "burst free" release system.

Table I is a summarization of the microsphere process description for preparing a peptide system (Histatin peptide) having a controlled release over the course of from 1 to 100 days.

Release profiles can be modified by a judicious blend of uncapped and capped polymers either in separate microspheres or in the same microspheres. Release from microcapsule formulations 1 through 21 listed in Table 1, occur independently of each other and hence the cumulative release from blends of these formulations are additive. By blending several formulations of uncapped and end-capped microspheres, release curves of any desired duration can be tailored. In addition, based on the release characteristics of uncapped and end-capped polymers,

blending of the two forms in a single formulation comprising different ratios of uncapped to capped polymer, would significantly influence the polymer hydration and hence release of the active core thereby providing release curves of any desirable pattern. Manipulation of polymer hydration and degradation resulting in modulation of release of active core is achieved by the addition of uncapped polymer to end-capped polymer in amounts as low as 1% up to 100%.

Table 1- Microcapsule compositions containing Histatin polypeptide

Composition#	Polymer Description			Theoretical peptide Core Load (%)	Internal Phase Ratio (w/o)	Emulsification Technique
	L/G Ratio & Type	Mol. Wt.(Mw x 10^3)	Conc. in DCM (w/w)			
1.	50/50 ,U	12	38	5	1:20	A
2.	50/50 ,U	12	18.5	2	1:20	A
3.	50/50	34	10	5	1:20	A
4.	50/50 ,U	12	38	5	1:4	A

5.	50/50	34	7	5	1:10	B
6.	50/50	34	10	5	1:10	B
7.	50/50	34	10	5	1:10	A
8.	75/25	12	10	5	1:10	B
9.	75/25	12	23.5	5	1:10	B
10.	50/50	12	10	5	1:10	B
11.	50/50	12	7	5	1:10	B
12.	50/50 ,U	12	10	5	1:10	B
13.	50/50 ,U	12	7	2.3	1:10	B

14.	50/50 ,U	12	10	5	1:10	B
15.	50/50 ,U	34	10	5	1:10	B
16.	50/50 ,U	12	10	5	1:10	B
17.	50/50 ,U	12	20	5	1:10	B
18.	50/50 ,U	12	40	5	1:10	B
19.	50/50 ,U	34	5	5	1:10	B
20.	50/50 ,U	34	10	5	1:10	B
21.	50/50 U	34	15	5	1:10	B

Acronyms:

L/G ratio: Copolymer composition of lactide/glycolide
DCM: Methylene Chloride
Mw: Molecular weight in daltons
A: w/o/w emulsification without an intermediate step for emulsion stabilization
B: w/o/w emulsification with an intermediate step for emulsion stabilization
U: Uncapped polymer

While referring to Table 1 in conjunction with FIG. 3, it can be seen that the cumulative Histatin release from PLGA microspheres from several batches prepared using 50/50 and 75/25 uncapped and end-capped, polymer modulates release between 1 to 100 days by varying the process parameters. 1-35 days by uncapped 50/50, 18-56 days by capped 50/50 and 56-100 days by capped 75/25.

In referring to FIG. 4, a view is provided through a scanning electron micrograph of PLGA microspheres designed for a one to two month release system prepared using end-capped polymer of Mw 30-40k daltons.

FIG. 5 depicts the cumulative Histatin release from PLGA microspheres, in which the release profiles are from several batches prepared using 50/50, uncapped and capped polymer, and varying the process parameters to modulate release between 28 to 60 days.

Figure 6 represents cumulative Histatin release from PLGA microspheres --- these combined release profiles are from several batches prepared using 50/50 uncapped and capped polymer, and varying the process parameters to modulate release between 1-60 days.

In the context of the invention, a biologically active agent is any water-soluble antibiotics, antitumor agents, antipyretics analgesics, anti-inflammatory agents, antitussives, expectorants, sedatives, muscle relaxants, anti epileptics, antiulcer agents,

anti-depressants, anti-allergic drugs, cardiotonics, antiarrhythmics drugs, vasodilators, antihypertensives, diuretics, anticoagulants, hormone drugs, anti-narcotics, etc.

In general, "burst free" sustained release delivery of biologically active agents from PLGA microshperes is accomplished in the context of this invention using of 90/10 to 40/60 molar ratios, and ratios of uncapped polymer to end-capped polymer of 100/0 to 1/99.

In general, the approaches for designing the biologically active agents encapsulated in the uncapped and combination uncapped/end-capped PLGA microspheres and characteristics of these encapsulants are briefly set forth below as follows:

1. Providing PLGA microspheres of surface morphologies using 50/50 uncapped and capped polymers of Mw ~ 8-40K daltons as shown in Figs. 2 and 4.

2. Providing in vitro release of a polypeptide, Histatin from PLGA microspheres, as shown in Figs. 3 and 5, using uncapped and capped polymer of Mw ~ 8-40K daltons and molar ratios such as 50/50 and 75/25.

For example, design of a 1-12 weeek bioactive compound release system is achieved using PLGA with the following specifications:

1. Polymer molecular weight:
   -about 2-60K daltons
2. Copolymer molar ratio (L/G):
   - 90/10 to 40/60
3. Polymer end groups:
   - uncapped and /or end-capped

and combining judiciously within the following parameters:

4. Polymer concentration
   - from 5 to 50%
5. Inner aqueous to oil phase ratio:
   - 1:5 to 1:20 (v/v)
6. Peptide loads:
   - from 2 to about 40% (w/w polymer)

and by using the unique aqueous emulsification method described in the invention.

The uniqueness and novelty of invention may generally be summarized in a brief way as follows:

1. Use of uncapped poly(lactide/glycolide) to achieve burst-free, continuous, sustained, programmable release of biologically active agents over 1-100 days.

2. Use of a unique aqueous emulsification system to achieve superior microsphere characteristics such as uniform sphere morphology and narrow size distribution.

3. Burst-free, prolonged, sustained release of polypeptides and other biologically actice agents from biocompatible and biodegradable microcapsules up to 100 days in an aqueous physiological environment without the use of additives in the inner core.

4. Release of active core programmable for variable durations over 1-100 days by using a blend of uncapped and capped polymer for different molecular weights and copolymer rations and manipulating the process parameters.

5. Complete release of the active core concurrent with complete solubilization of carrier polymer to innocuous components such as lactic and glycolic acids, especially when using a 100/0 blend of uncapped/capped polymer. This is of tremendous significance as most biodegradable polymers currently in use for 1-30 day delivery, do not degrade completely at the end of the intended release duration causing serious concern for regulatory authorities on the effects of residual polymer at the site of administration.

6. Ease of administration of the microcapsules in various dosages forms via several routes such as parenteral (intramusclar and subcutaneous), oral, topical, nasal, vaginal, etc.

The following examples are illustrative of, but not limitations upon the microcapsule compositions pertaining to this invention.

Example 1

Polylactide/glycolide (PLGA) microcapsules are prepared by a unique aqueous emulsification technique which has been developed for use with the uncapped polymer to provide superior sphere morphology, sphere integrity and narrow size distribution (See Figures 1a and 1b). This is accomplished by dissolving the polymer in a chlorinated hydrocarbon solvent such as methylene chloride and dissolving the biologically active agent in water. A

w/o emulsion is then formed by mixing the solutions of polymer and the active agent by sonication, followed by emulsion stabilization in a solvent - saturated aqueous solution containing polyvinyl alcohol. A ternary emulsion is then formed by emulsifying the w/o emulsion in an external, pre-cooled aqueous phase containing polyvinyl alcohol (0.25 - 1% w/v). Microcapsules are hardened upon removal of solvent by evaporation, rinsed to remove any residual emulsifier, and then lyophilized.

Table 1 lists the microcapsule compositions, Nos. 1-21 thus prepared, consisting of a biologically active polypeptide, Histatin (composed of 12 amino acids and a molecular weight of 1563) and blends of uncapped and capped polymer of ratios 100/0 to 1/99, and having a lactide/glycolide ratio of 90/10 to 40/60, and a molecular weight range between 2000 to 60,000 daltons.

Example 2

Microcapsule compositions are prepared as described in Example 1 wherein the copolymer L/G ratio is 48/52 to 52/48, and the ratio of uncapped/capped polymer is 100/0. The active core is Histatin (Mw 1563), the polymer molecular weight is < 15,000 and the polymer concentrations vary from 7% to ~ 40% w/w. Compositions 1,2,4 12-14 and 16-18 are listed in Table 1.

Release profiles of the active core from the compositions in an aqueous physiological environment, such as phosphate-buffered saline, pH 7.0 maintained at 37 ± 1°C are plotted as cumulative percentage release versus time, and presented in Figure 2.

Burst-free, variable release from 1-35 days is achieved by varying the polymer concentration from 7 to ~ 40% w/w in the oil phase.

Example 3

Microcapsule compositions are prepared as described in Example 2, wherein the aqueous /oil ratio is varied from 1/4 to 1/20 (v/v). Compositions 1,2,4 and 12 are listed in Table 1.

Release profiles of the active core from the compositions in an aqueous physiological environment described in Example 2 are plotted as cumulative percentage release versus time, and presented in Figure 2.

Burst-free, continuous release from 1-35 days, with different onset and completion times are achieved by selecting

different w/o ratios in the inner core.

Example 4

Microcapsule compositions are prepared as described in Example 2, wherein the polymer molecular weight is 28,000-40,000 and polymer concentrations vary from 5% to ~ 15% w/w. Compositions 19-21 are listed in Table 1.

Release profiles of the active core from the compositions in an aqueous physiological environment are described in Example 2 are plotted as cumulative percentage release versus time and presented in Figure 3.

Burst-free, variable release from 18-40 days is achieved by varying the polymer concentration.

Example 5

Microcapsule compositions are prepared as described in Example 2, wherein the ratio of uncapped/capped polymer is 1/99 and polymer concentrations vary between 5% to ~ 12% w/w. Compositions 10 and 11 are listed in Table 1.

Release profiles of the active core from the compositions in an aqueous physiological environment are described in Example 2, and plotted as cumulative percentage release versus time and presented in Figure 2.

Burst-free, variable release from 28-70 days is achieved by varying the polymer concentration in the oil phase.

Example 6

Microcapsule compositions are prepared as described in Example 5, wherein polymer molecular weight is 28,000-40,000 and polymer concentrations vary between 5% to ~ 12% w/w. Compositions 5 and 6 are listed in Table 1.

Release profiles of the active core from the compositions in an aqueous physiological environment are described in Example 2 and are plotted as cumulative percentage release versus time, and presented in Figure 3.

Burst-free, variable release from 28-70 days is achieved by varying the polymer concentration.

Example 7

Microcapsule compositions are prepared as described in Example 6, wherein the aqueous/oil ratio varies between 1/5 to

1/25 (v/v). Compositions 3 and 7 are listed in Table 1.

Release profiles of the active core from the compositions in an aqueous physiological environment are described in Example 2, and plotted as cumulative percentage release versus time, and presented in Figure 3.

Burst-free, variable release from 28-70 days is achieved by varying the aqueous/oil ratios.

Example 8

Microcapsule compositions are prepared as described in Example 5, wherein the copolymer ratio is 75/25 and polymer concentrations vary between 5% to ~ 25% w/w. Compositions 8 and 9 are listed in Table 1.

Release profiles of the active core from the compositions in an aqueous physiological environment are described in Example 2, and are plotted as cumulative percentage release versus time, and presented in Figure 2.

Burst-free, variable release from 56->90 days is achieved by varying the polymer concentration in the oil phase.

Example 9

Microcapsule compositions are described in Example 2, wherein the active core is leutinizing hormone releasing hormone (LHRH, a decapeptide of molecular weight 1182) and the polymer concentration is ~40% w/w. Release profiles of the active core from the composition in an aqueous physiological environment is described in Example 2, and is plotted as cumulative percentage release versus time, and presented in Figure 4.

Burst-free, continuous and complete release is achieved within 35 days, similar to Histatin acetate.

Example 10

Microcapsule compositions are prepared as described in Example 2, wherein an additive such as sodium salt (carbonate or bicarbonate) is added to the inner aqueous phase at concentrations of 1-10% w/w to maintain the biological activity of the released polypeptide.

Burst-free, variable release from 1-28 days is achieved similar to Examples 2 & 3, and the released polypeptide is biologically active until 30 days, due to the presence of the sodium salt.

Example 11

Microcapsule compositions are prepared as described in Example 2, wherein an additive such as a nonionic surfactant, polyoxyethylene/polyoxypropylene block copolymer (Pluronics F68 and F127) is added to either the inner oil or the aqueous phase at concentrations from 10-100% w/w, to maintain the biological activity of the released polypeptide.

Burst-free, continuous release from 1-35 days is achieved similar to Examples 2 & 3, and the released polypeptide is bioactive due to the presence of the surfactant.

Example 12

Cumulative histatin release from the microcapsule compositions described in Examples 1 through 11 and release profiles plotted in Figures 2 and 3 show the burst-free, programmable peptide release for variable duration from 1-100 days. Virtually any pattern of cumulative release is achievable over a 100 day duration by a judicious blending of several compositions, as shown in these figures.

WE CLAIM

1. A controlled release microcapsule pharmaceutical formulation for burst-free, sustained, programmable release of a biologically active agent over a duration from 1-100 days, comprising an active agent and a blend of uncapped and end-capped biodegradable poly(lactide/glycolide).

2. The pharmaceutical formulation of claim 1, wherein the biodegradable poly(lactide/glycolide) is a blend of uncapped and capped forms, in ratios ranging from 100/0 to 1/99.

3. The microcapsules of claims 1 or 2 wherein the copolymer (lactide to glycolide L/G) ratio for uncapped and endcapped polymer is 52/48 to 48/52.

4. The microcapsules of claims 1 or 2 wherein the copolymer L/G ratio for uncapped and end-capped polymer is 90/10 to 40/60.

5. The microcapsules of claim 3 wherein the molecular weight of the copolymer is between 2,000-60,000 daltons.

6. The microcapsules of claim 1 wherein the biologically active agent is a peptide or polypeptide.

7. The microcapsules of claim 6, wherein said polypeptide is histatin consisting of 12 amino acids and having a molecular weight of 1563.

8. The microcapsules of claim 7 characterized by the capacity to completely release histatin in an aqueous physiological environment from 1-35 days with a 100/0 blend of uncapped and end-capped poly(lactide/glycolide) having a L/G

ratio of 48/52 to 52/48, and a molecular weight <15,000.

9. The microcapsules of claim 7 characterized by the capacity to completely release histatin in an aqueous physiological environment from 18-40 days with a 100/0 blend of uncapped and end-capped poly(lactide/glycolide) having a L/G ratio of 48/52 to 52/48 and a molecular weight range of 28,000-40,000.

10. The microcapsules of claim 7 characterized by the capacity to release up to 90% of the histatin in an aqueous physiological environment from 28-70 days with a 0/100 blend of uncapped and end-capped poly(lactide/glycolide) having a L/G ratio of 48/52 to 52/48 and a molecular weight range of 10,000-40,000 daltons.

11. The microcapsules of claim 7 characterized by the capacity to release up to 80% of histatin in an aqueous physiological environment from 56-100 days with a 0/100 blend of uncapped and end-capped poly(lactide/glycolide) having a L/G ratio of 75/25 and a molecular weight of < 15,000 daltons.

12. The microcapsules of claims 7 or 8 or 9 or 10 or 11 having analogs of histatin with chain lengths of from 11-24 amino acids of molecular weights from 1,500-3,000 daltons and characterized by the following structures:

1. D S H A K R H H G Y K R K F H E K H H S H R G Y
2. K R H H G Y K R K F H E K H H S H R G Y R
3. K R H H G Y K R K F H E K H H S H R
4. R K F H E K H H S H R G Y R

5. A K R H H G Y K R K F H

6. *A K R H H G Y K R K F H

7. K R H H G Y K R K F

* D-amino acid

13. The microcapsules of claim 6 wherein the biologically active agent is a polypeptide Leutinizing hormone releasing hormone (LHRH) that is a decapeptide of molecular weight 1182 in its acetate form, and having the structure:

p- E H W S Y G L R P G

14. The microcapsule of claim 6 having a molecular weight of from 1,000 to 250,000 daltons.

15. The microcapsules of claims 6 or 7 or 8 or 9 or 10 or 11 or 13 or 14 wherein release profiles of variable rates and durations are achieved by blending uncapped and capped microspheres as a cocktail in variable amounts.

16. The microcapsules of claims 6 or 7 or 8 or 9 or 10 or 11 or 13 or 14 wherein release of profiles of variable rates and duration are achieved by blending uncapped and capped polymer in different ratios within the same microshreres.

17. The microcapsules of claims 6 or 7 or 8 or 9 or 10 or 11 or 13 or 14 wherein the entrapped polypeptide is any of the vaccine agents against enterotoxigenic E. coli (ETEC) such as CFA/I,CFA/II,CS1,CS3,CS6 and CS17 and other ETEC-related enterotoxins.

18. The microcapsules of claims 6 or 7 or 8 or 9 or 10 or 11 13 or 14 wherein the entrapped polypeptide consists of peptide antigens of molecular weight range of about 800-5000 daltons for immunization against enterotoxigenic E. coli (ETEC).

19. The microcapsules of claims 1 or 2 or 3 or 4 or 5 wherein said biologically active agents are selected from the group consisting of water-soluble hormone drugs, antibiotics, antitumor agents, anti inflammatory agents, antipyretics, analgesics, antitussives, expectorants, sedatives, muscle relaxants, antiepileptics, antiulcer agents, antidepressants, antiallergic drugs, cardiotonics, antiarrhythmic drugs, vasodilators, antihypertensives, diuretics, anticoagulants, and antinarcotics, in the molecular weight range of 100-100,000 daltons.

20. The microcapsules of claims 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 wherein said biodegradable poly(lactide/glycolide) is in an oil phase, and is present in about 1-50% (w/w).

21. The microcapsules of claims 1 or 2 or 3 or 4 or 5 or 6 or 8 or 9 or 10 or 11 or 13 or 14 wherein concentration of the active agent is in the range of 0.1 to about 60% (w/w).

22. The microcapsules of claims 1 or 2 or 3 or 4 or 5 or 6 or 8 or 9 or 10 or 11 or 13 or 14 wherein a ratio of the inner aqueous to oil phases is about 1/4 to 1/40(v/v).

23. A process for preparing controlled release microcapsule formulations characterized by burst-free, sustained, programmable release of biologically active agents comprising: Dissolving

biodegradable poly (lactide/glycolide), in uncapped form in methylene chloride, and dissolving a biologically active agent or active core in water; adding the aqueous layer to the polymer solution and emulsifying to provide an inner water-in-oil (w/o) emulsion; stabilizing the w/o emulsion in a solvent-saturated aqueous phase containing a oil-in-water (o/w) emulsifier; adding said w/o emulsion to an external aqueous layer containing oil-in-water emulsifier to form a ternary emulsion; and stirring the resulting water-in-oil-in-water (w/o/w) emulsion for sufficient time to remove said solvent, and rinsing hardened microcapsules with water and lyophilizing said hardened microcapsules.

24. A process for preparing controlled release microcapsule formulations characterized by burst-free, sustained, programmable release of biologically active agents comprising:

dissolving biodegradable poly(lactide/glycolide) in end-capped form in methylene chloride, and dissolving a biologically active agent or active core in water; adding the aqueous layer to the polymer solution and emulsifying to provide an inner water-in-oil emulsion; stabilizing the w/o emulsion in a solvent-saturated aqueous phase containing a oil-in-water (o/w) emulsifier; adding said w/o emulsion to an external aqueous layer containing oil-in-water emulsifier to form a ternary emulsion; and stirring a resulting water-in-oil-water (w/o/w) emulsion for sufficient time to remove said solvent; and rinsing hardened microcapsules with water; and lyophilizing said hardened microcapsules.

25. The process of claims 23 or 24 wherein a solvent-saturated external aqueous phase is added to emulsify the inner w/o emulsion prior to addition of the external aqueous layer, to provide microcapsules of narrow size distribution range between 0.05-500μm.

26. The process of claims 23 or 24, wherein a low temperature of about 0-4°C is provided during preparation of the inner w/o emulsion, and a low temperature of about 4-20°C is provided during preparation of the w/o/w emulsion to provide a stable emulsion and high encapsulation efficiency.

27. The process of claim 23 wherein a 100/0 blend of uncapped and end-capped polymer is used to provide release of the active core in a continous and sustained manner without a lag phase.

28. The microcapsules of claim 6, wherein, when the entrapped polypeptide is active at a low pH, such as LHRH, adrenocorticotropic hormone, epidermal growth factor, calcitonin released polypeptide is bioactive.

29. The microcapsules of claims 6 or 7 or 8 or 9 or 10 or 11, wherein, when entrapped peptide such as histatin is inactive at a low pH, a pH-stabilizing agent of inorganic salts are added to the inner aqueous phase to maintain biological activity of the released peptide.

30. The microcapsules of claims 6 or 7 or 8 or 9 or 10 or 11 wherein, when entrapped polypeptide such as histatin is inactive at a low pH, a non-ionic surfactant such as polyoxyethylene

sorbitan fatty acid esters (Tween 80, Tween 60 and Tween 20) and polyoxyethylene - polyoxypropylene block copolymers (Pluronics) is added to the inner aqueous phase to maintain biological activity of the released polypeptide.

31. The microcapsules of claim 29, wherein placebo spheres loaded with the pH-stabilizing agents are coadministered with polypeptide-loaded spheres to maintain the solution pH around the microcapsules and preserve the biological activity of the released peptide in instances where the addition of pH-stabilizing agents in the inner aqueous phase is undesirable for the successful encapsulation of the acid pH sensitive polypeptide.

32. The microcapsules of claim 30 wherein placebo spheres loaded with non-ionic surfactant are coadministered with polypeptide-loaded spheres to maintain biological activity of the released peptide where the addition of non-ionic surfactants in the inner aqueous phase is undesirable for successful encapsulation of the acid pH sensitive polypeptide.

33. The microcapsules of claims 1 or 2 or 3 or 4 or 5 or 6 or 8 or 9 or 10 or 11 or 13 or 14 comprising a blend of uncapped and capped polymer, wherein complete solubilization of the copolymer leaves no residual polymer at the site of administration and occurs concurrently with the complete release of the entrapped agent.

34. A process of using microcapsules of claims 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 13 or 14 for human

administration via parenteral routes, such as intramuscular and subcutaneous.

35. A process of using microcapsules of claims 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 13 or 14 for human administration via topical route.

36. A process of using microcapsules of items 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 13 or 14 for human administration via oral routes.

37. A process of using microcapsules of items 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 13 or 14 for human admininstration via nasal, transdermal, rectal, and vaginal routes.

Abstract

Novel burst-free, sustained release biocompatible and biodegrable microcapsules which can be programmed to release their active core for variable durations ranging from 1-100 days in an aqueous physiological environment. The microcapsules are comprised of a core of polypeptide or other biologically active agent encapsulated in a matrix of poly(lactide/glycolide) copolymer as a blend of uncapped (free carboxyl end group) and end-capped forms ranging in ratios from 100/0 to 1/99.

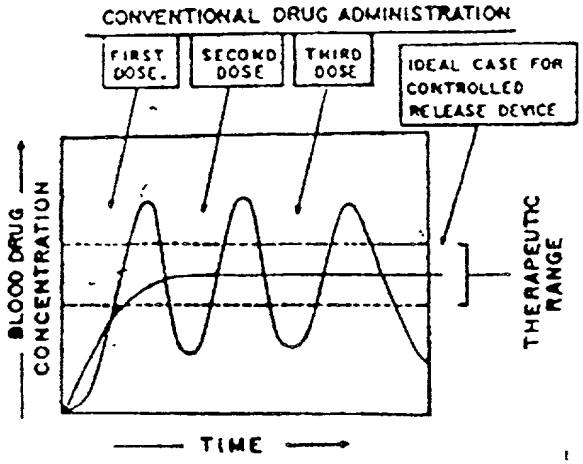

CONVENTIONAL DRUG ADMINISTRATION
FIRST DOSE.
SECOND DOSE
THIRD DOSE
IDEAL CASE FOR CONTROLLED RELEASE DEVICE
BLOOD DRUG CONCENTRATION
THERAPEUTIC RANGE
TIME

Fig. 1

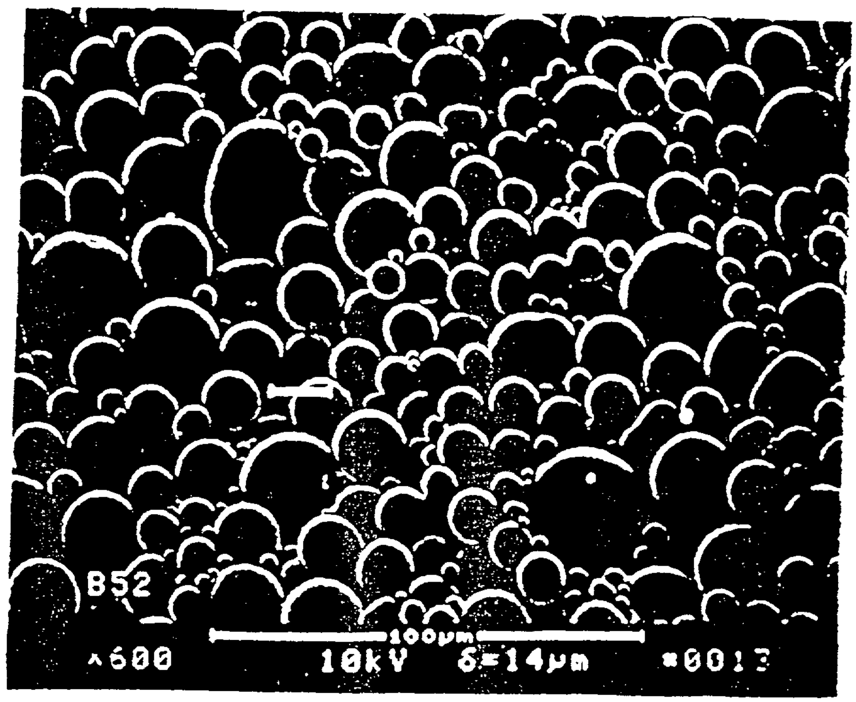


Fig. 2

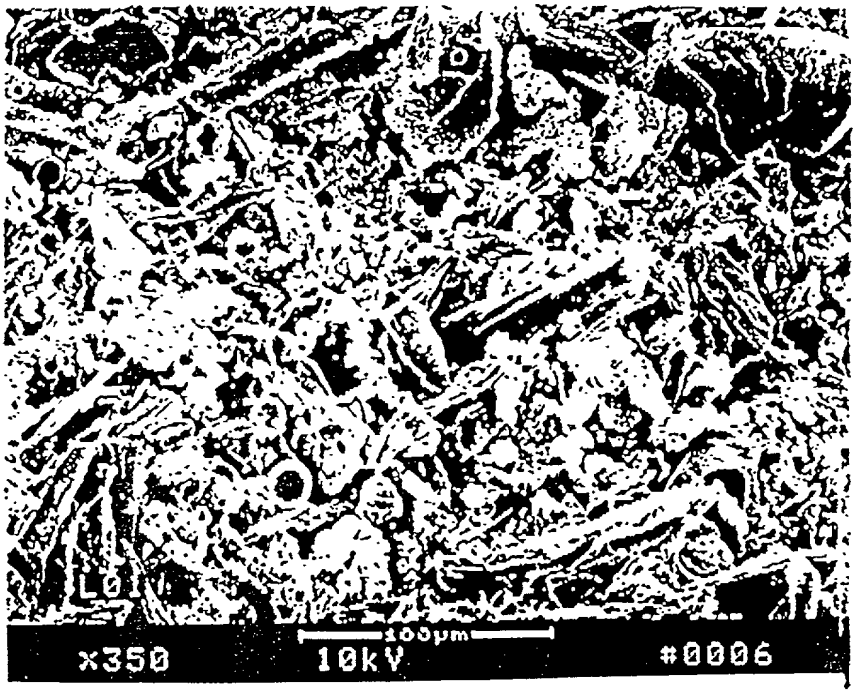


Figure 2a

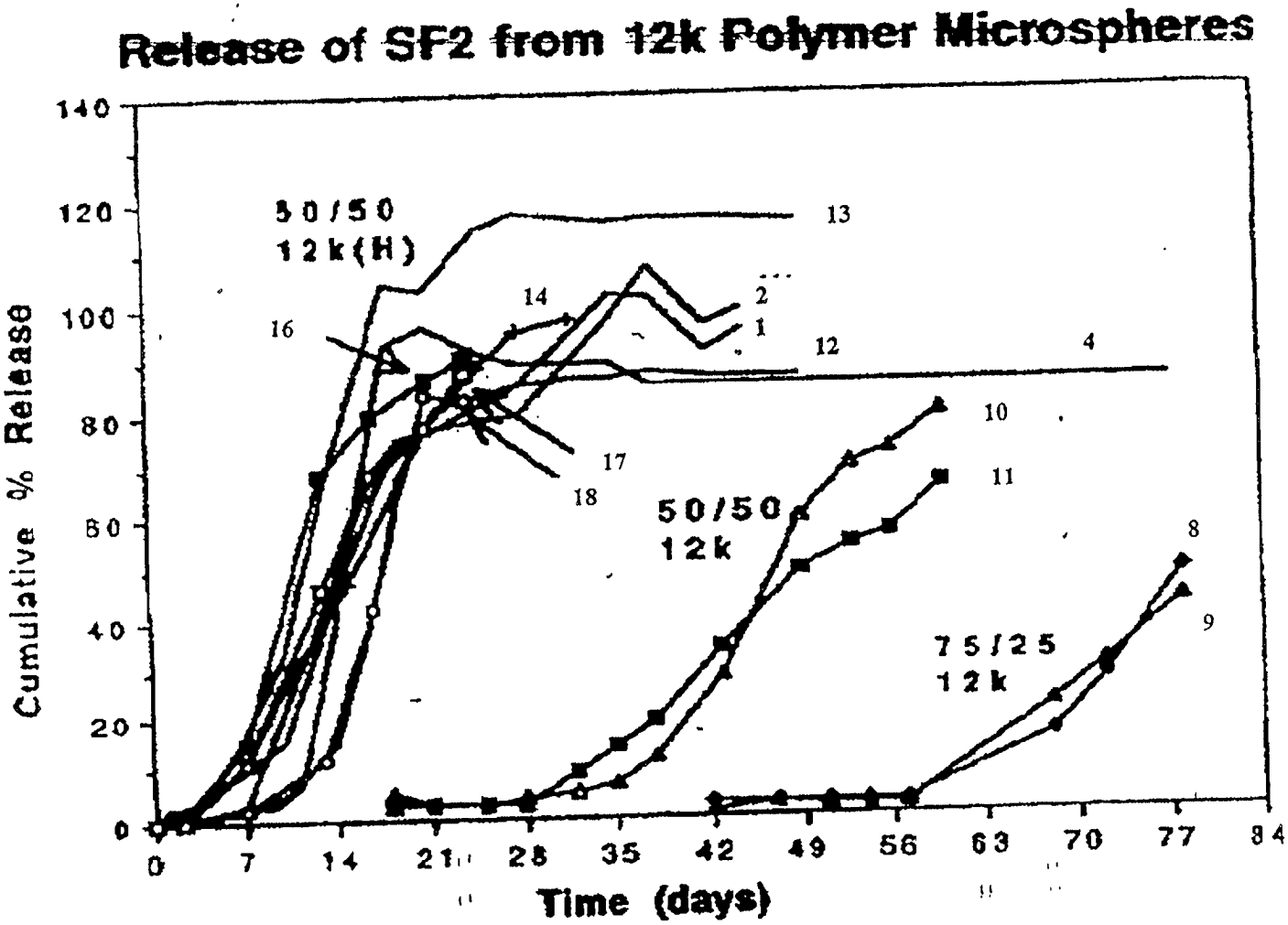


Fig.3

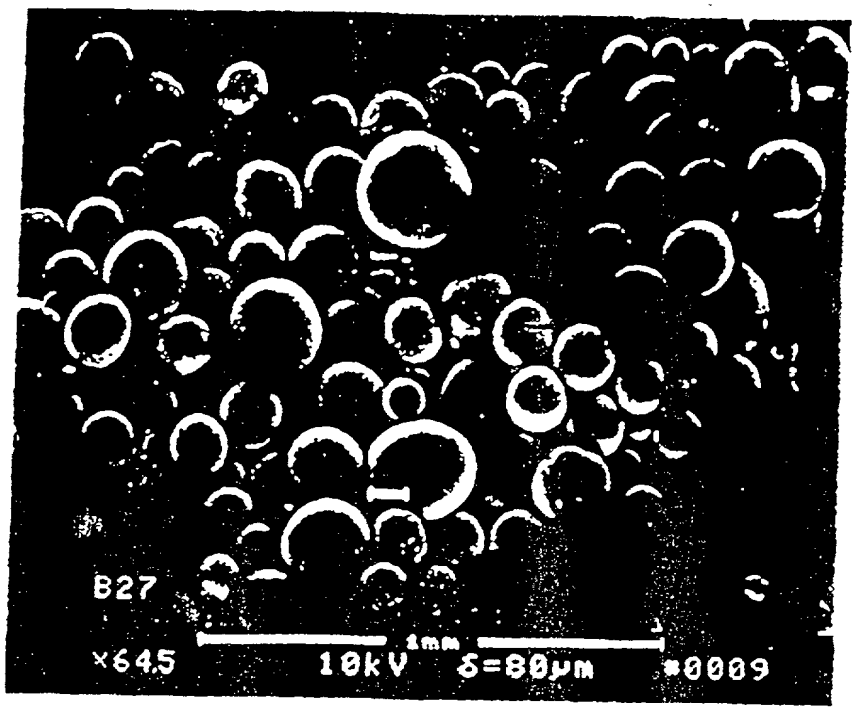


Fig. 4

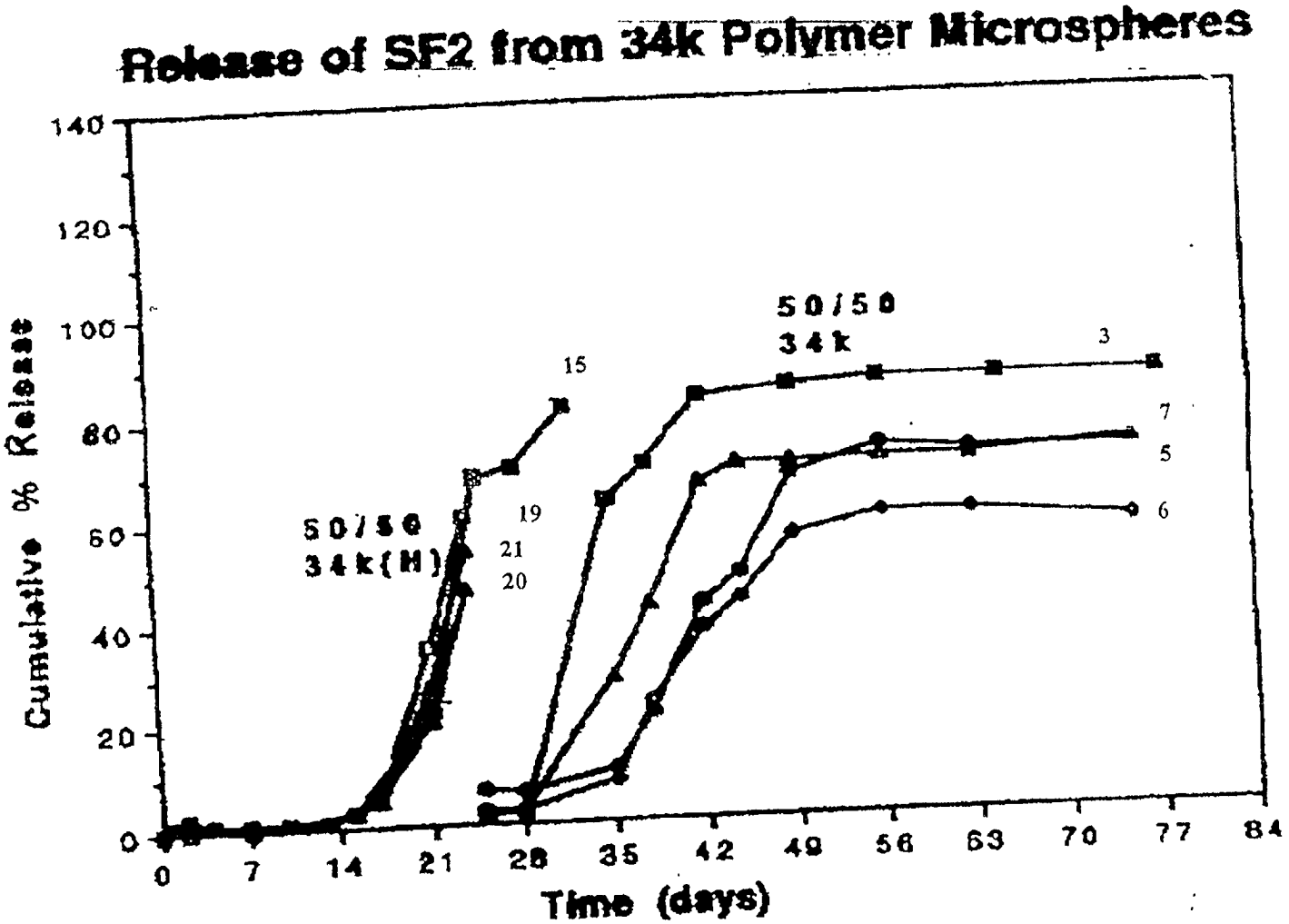


Fig. 5

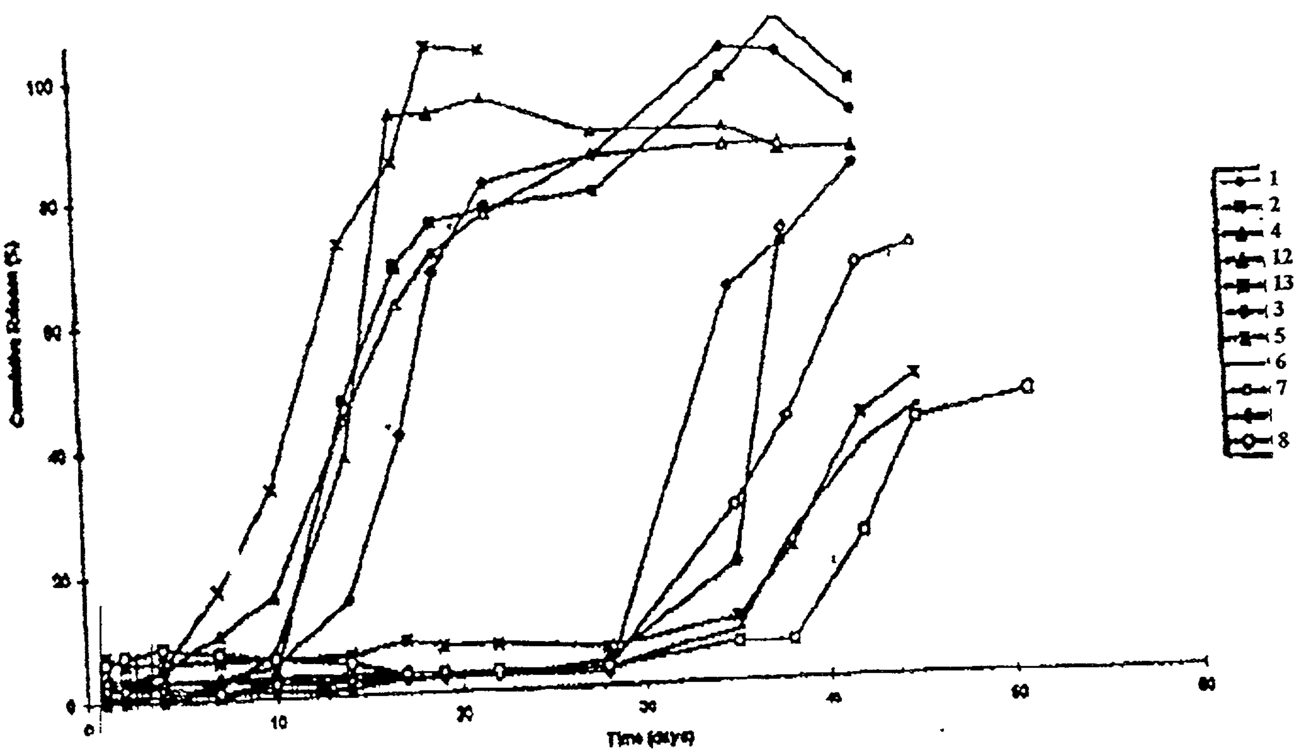


Fig. 6

Sheet1 Chart 1

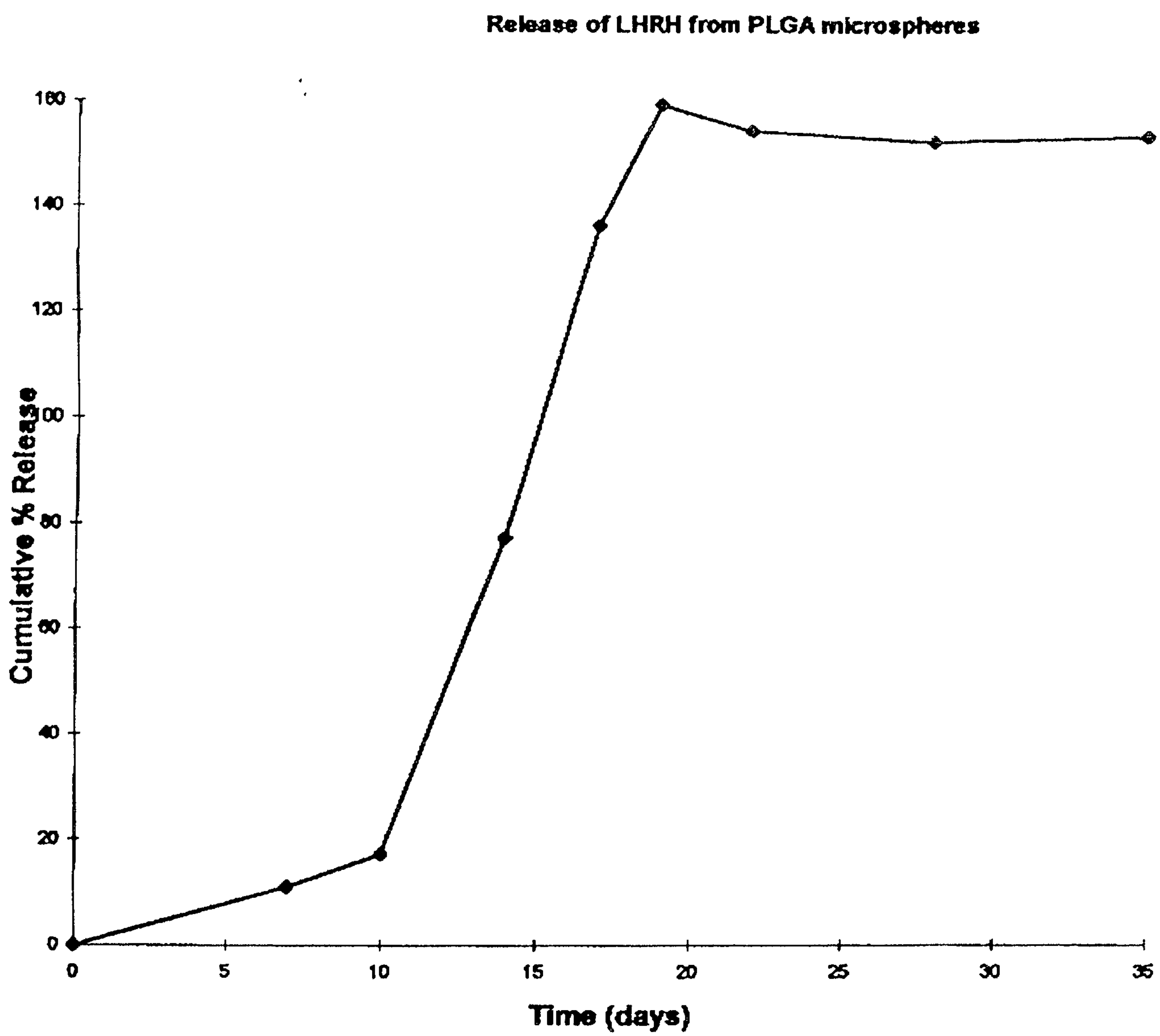


Figure 7

Associate Power Of Attorney Or Agent (37 CFR 1.34) (For Representation Related To A Patent Application)	Docket No. Army 111

In Re Application Of: Jeyanthi, et al.

Serial No.	Filing Date	Examiner	Group Art Unit
08/598,973	January 24, 1996	Harrison, R.	1626

Invention: Novel Burst-Free Sustained Release Poly(Lactide/Glycolide)

TO THE ASSISTANT COMMISSIONER FOR PATENTS:

Please recognize the following as ☒ Associate Attorney ☐ Associate Agent In this application.

Name: Mariana Titus and Caroline Nash

Reg. No.: 35,843 and 36,329, respectively

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Signature of Principal Attorney or Agent of Record

Dated: 5/6/99

Wertes F.W. Bellamy, Reg. No. 27, 029
Intellectual Property Law Division
OTJAG, Department of the Army
901 North Stuart Street
Arlington, VA 22203-1837

Registration Number & Address of Principal Attorney or Agent of Record

I certify that this document is being deposited on 5-11-99 with the U.S. Postal Service as first class mail under 37 C.F.R. 1.8 and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

Caroline Nash

Signature of Person Mailing Correspondence

Caroline Nash

Typed or Printed Name of Person Mailing Correspondence

DECLARATION FOR PATENT APPLICATION X

Docket Number (Optional)

As a below named inventor, I hereby declare that: Ramasubbu Jeyanthi, John E. n Hamont, Phil Friden, Robert H. Reid, F. Donald Robe..s, Charles E. McQueen, Jean A. Petterstrom

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled Novel "Burst-Free" Sustained Release Poly(Lactide/Glycolide) Microspheres, the specification of which is attached hereto unless the following box is checked:

[x] was filed on 1/24/96 as United States Application Number or PCT International Application Number 08/590,973 and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)			Priority Claimed
(Number)	(Country)	(Day/Month/Year Filed)	☐ Yes ☐ No
(Number)	(Country)	(Day/Month/Year Filed)	☐ Yes ☐ No
(Number)	(Country)	(Day/Month/Year Filed)	☐ Yes ☐ No

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

(Application Number)	(Filing Date)	(Status – patented, pending, abandoned)
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I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: Earl T. Reichert, Reg #24,331; Werten F.W. Bellamy Reg #27,029; William W. Randolph, Reg #28,986; Maj Murray B. Baxter, Reg #33,646

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ARLINGTON, VA 22203-1837

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of first inventor (given name, family name) Ramasubbu Jeyanthi

first inventor signature _____ Date 05/24/96

Residence 9725 CLOCKTOWER LANE #301 COLUMBIA MD 21046 Citizenship INDIA

Post Office Address 9725 CLOCKTOWER LANE APT. #301
COLUMBIA MD 21046

Full name of joint inventor, if any (given name, family name) _____

Date _____

Residence _____ Citizenship _____

Post Office Address _____

[xx] Additional inventors are being named on separately numbered sheets attached hereto.

DECLARATIO FOR PATENT APPLICATION

Docket Number (Optional)

As a below named inventor, I hereby declare that: Ramasubbu Jeyanthi, John E. an Hamont, Phil Friden, Robert H. Reid, F. Donald Roberts, Charles E. McQueen, Jean A. Setterstrom

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Full name of second inventor (given name, family name) John E. Van Hamont — Date 13 MAY 1996

Residence 7446 VAN NOY LOOP, FORT MEADE, MD. 20755 — Citizenship USA

Post Office Address 7446 VAN NOY LOOP, FORT MEADE, MD. 20755

Full name of second joint inventor, if any (given name, family name) _____ — Date _____

Residence _____ — Citizenship _____

Post Office Address _____

[xx] Additional inventors are being named on separately numbered sheets attached hereto. page 2 of 7

DECLARATION FOR PATENT APPLICATION

Docket Number (Optional)

As a below named inventor, I hereby declare that: Ramasubbu Jeyanthi, John E. Mont, Phil Friden, Robert H. Reid, F. Donald Roberts, Charles E. McQueen, Jean A. Setterstrom

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I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled Novel "Burst-Free" Sustained Release Poly(Lactide/Glycolide) Microspheres, the specification of which

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Full name of third inventor (given name, family name) Phil Friden

third inventor signature _____ Date 5/23/96

Residence [redacted] Citizenship US

Post Office Address 32 Washington Street
Bedford, MA 01730

Full name of joint inventor, if any (given name, family name) _____

_____ Date _____

Residence _____ Citizenship _____

Post Office Address _____

[X] Additional inventors are being named on separately numbered sheets attached hereto.

DECLARATION FOR PATENT APPLICATION

Docket Number (Optional)

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of fourth inventor (given name, family name) Robert H. Reid Robert H. Reid Date 5/28/96

Residence 48 Lake Side Tr. C.V., Fairfield, PA, 17320 Citizenship USA

Post Office Address P.O. Box 531
Fairfield, PA 17320

Full name of second joint inventor, if any (given name, family name) _____ Date _____

Residence _____ Citizenship _____

Post Office Address _____

[xx] Additional inventors are being named on separately numbered sheets attached hereto. page 4 of 7

DECLARATION FOR PATENT APPLICATION

Docket Number (Optional)

As a below named inventor, I hereby declare that: Ramasubbu Jeyanthi, John E. Lamont, Phil Friden, Robert H. Reid, F. Donald Roberts, Charles E. McQueen, Jean A. Setterstrom

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled _____, the specification of which

is attached hereto unless the following box is checked:

[X] was filed on 1/24/96 as United States Application Number or PCT International Application Number 08/590,973 and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)			Priority Claimed
(Number)	(Country)	(Day/Month/Year Filed)	☐ Yes ☐ No
(Number)	(Country)	(Day/Month/Year Filed)	☐ Yes ☐ No
(Number)	(Country)	(Day/Month/Year Filed)	☐ Yes ☐ No

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

(Application Number)	(Filing Date)	(Status – patented, pending, abandoned)
(Application Number)	(Filing Date)	(Status – patented, pending, abandoned)

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: Earl T. Reichert, Reg #24,331; Werten F.W. Bellamy, Reg #27,029; William W. Randolph, Reg #28,986; Maj Murray B. Baxter, Reg #33,646

Address all telephone calls to _____ at telephone number _____

Address all correspondence to INTELLECTUAL PROPERTY LAW DIVISION
OFFICE OF THE JUDGE ADVOCATE GENERAL, DA
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ARLINGTON, VA 22203-1837

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Full name of fifth inventor (given name, family name) F. Donald Roberts

fifth inventor signature F. Donald Roberts Date MAY 23, 1996

Residence 2 BRIDLE PATH CIRCLE Citizenship U.S.

Post Office Address DOVER, MA 02030

Full name of sixth joint inventor, if any (given name, family name) Charles E. McQueen

_____ Date _____

Residence _____ Citizenship _____

Post Office Address _____

[X] Additional inventors are being named on *separately numbered sheets* attached hereto. page 5 of 7

DECLARATIO'' FOR PATENT APPLICATION

Docket Number (Optional)

As a below named inventor, I hereby decla. .iat: Ramasubbu Jeyanthi, John E. .n Hamont, Phil Friden, Robert H. Reid, F. Donald Roberts, Charles E. McQueen, Jean A. Setterstrom

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled Novel "Burst-Free" Sustained Release Poly(Lactide/Glycolide) Microspheres, the specification of which

is attached hereto unless the following box is checked:

[x] was filed on 1/24/96 as United States Application Number or PCT International Application Number 08/590,973 and was amended on _____ (if applicable).

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Prior Foreign Application(s)			Priority Claimed
(Number)	(Country)	(Day/Month/Year Filed)	☐ Yes ☐ No
(Number)	(Country)	(Day/Month/Year Filed)	☐ Yes ☐ No
(Number)	(Country)	(Day/Month/Year Filed)	☐ Yes ☐ No

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

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Full name of sixth inventor (given name, family name) Charles E. McQueen — Date 10 May 96

Residence 16805 Ethelwood Ter — Citizenship USA

Post Office Address Olney MD 20832

Full name of second joint inventor, if any (given name, family name) _____ — Date _____

Residence _____ — Citizenship _____

Post Office Address _____

[xx] Additional inventors are being named on separately numbered sheets attached hereto. page 6 of 7

DECLARATION FOR PATENT APPLICATION

Docket Number (Optional)

As a below named inventor, I hereby decla[illegible]nat: Ramasubbu Jeyanthi, John E. [illegible]amont, Phil Friden, Robert H. Reid, F. Donald Roberts, Charles E. McQueen, Jean A. Setterstrom
My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled _____, the specification of which

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(Number)	(Country)	(Day/Month/Year Filed)	☐ Yes ☐ No
(Number)	(Country)	(Day/Month/Year Filed)	☐ Yes ☐ No
(Number)	(Country)	(Day/Month/Year Filed)	☐ Yes ☐ No

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Full name of seventh inventor (given name, family name) Jean A. Setterstrom
signature Jean A. Setterstrom Date 9 May 1996
Residence 407 Boston Ave Citizenship USA
Post Office Address Silver Spring, MD 20913

Full name of joint inventor, if any (given name, family name) _____
inventor signature _____ Date _____
Residence _____ Citizenship _____
Post Office Address _____

[X] Additional inventors are being named on separately numbered sheets attached hereto. page 7 of 7